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Pulsatile drug release from poly (lactide-*co*-glycolide) microspheres: how does the composition of the polymer matrices affect the time interval between the initial burst and the pulsatile release of drugs?

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Abstract

Pulsatile release of estradiol was observed from poly (lactide-*co*-glycolide) microspheres, of which the monomer composition was 75% lactide and 25% glycolide. Estradiol was monolithically dissolved in the polymer matrices. The microspheres were immersed in a pH 7.4 phosphate buffer saline at 37°C. When estradiol was loaded in microspheres consisting of poly (lactide-*co*-glycolide) of average molecular weight ($\overline{M}$w) of 74 000 before degradation, the pulse of estradiol release was observed almost 50 days after the initial burst. On the other hand, if poly (lactide-*co*-glycolide) of $\overline{M}$w 44 000 before degradation was used as a material to prepare the microspheres, then estradiol was released in a pulsatile manner almost 20 days after the initial burst effect. It was found that the time interval between the initial burst and the pulsatile release can be regulated by mixing the above two types of poly (lactide-*co*-glycolide) with different $\overline{M}$w to prepare microspheres. For example, the pulsatile release of estradiol was observed 30 days after the degradation starts when the microspheres were composed of 50% poly (lactide-*co*-glycolide) of $\overline{M}$w 74 000 and 50% poly (lactide-*co*-glycolide) of $\overline{M}$w 44 000. In another case where the microspheres were composed of 75% poly (lactide-*co*-glycolide) of $\overline{M}$w 74 000 and 25% poly (lactide-*co*-glycolide) of $\overline{M}$w 44 000, the pulsatile release was observed 38 days after the degradation starts.

Keywords: Pulsatile release; Poly (lactide-*co*-glycolide); Microsphere; Biodegradable polymer

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1. Introduction

A number of studies have been made on drug release from biodegradable polymers [1–4]. In particular, the drug release rate from the matrices composed of poly (lactide-*co*-glycolide), abbreviated to PLGA hereafter, is reported to be controlled by both the diffusion rate of drugs in the matrices and the degradation rate of the matrices [5].

In a previous paper [6], we have shown that sustained release of 17β-estradiol from poly (lactide-*co*-glycolide) microspheres containing 0.3% (w/w) estradiol was observed for 30 days and the release rate was kept almost constant. In the study, we have used poly (lactide-*co*-glycolide), the monomer composition of which was 50% lactide and 50% glycolide. Also, it was reported [6] that the release of estradiol from poly (lactide-*co*-glycolide) microspheres was affected by the following three mechanisms, (i) the release of estradiol molecules accompanied with the removal of degraded short polymer chains, (ii) the dissolution of estradiol crystals into polymer matrices and aqueous solution, (iii) pore formation in polymer matrices caused by the polymer degradation. It has been reported that the zeroth order release of drugs are available from poly (lactide-*co*-glycolide) with relatively low molecular weight [7,8].

In 1996, Robert Langer and his colleagues have reported pulsatile release of tetanus toxoid from poly (DL-lactide-*co*-glycolide) microcapsules and its application to vaccination [9]. They have prepared two types of microcapsules using poly (DL-lactide-*co*-glycolide) with different monomer compositions and average molecular weights. These microcapsules are hydrolytically degraded into monomers or oligomers with different degradation rates, because poly (DL-lactide-*co*-glycolide) used to prepare each type of microcapsules has different monomer compositions and molecular weights. They have shown [9] that a pulsed release of drugs occurs 3 and 7 weeks after the degradation starts from two types of microcapsules with different degradation rates, respectively. In their work, it was considered that the pulsed release was controlled successfully by the degradation rates of each type of poly (DL-lactide-*co*-glycolide), since the microcapsules contained mineral oil and tetanus toxoid was kept in the oil and no release was possible before channels were formed in the microcapsule wall between the microcapsule core and the outside of the microcapsule by hydrolytic degradation.

In the present study, we will show that the pulsatile release of estradiol, which is little soluble in water but soluble in PLGA matrices, occurs from PLGA microspheres. We will discuss the mechanisms which control the release rate of estradiol. Also, we will prepare four types of microspheres using two types of PLGA of $\overline{M}$w 74 000 and $\overline{M}$w 44 000, respectively. One of our challenges is to control the time interval between the initial burst and the pulsatile release of drugs. For this purpose, we will prepare microspheres composed of a mixture of the two types of PLGA with different $\overline{M}$w.

2. Experimental

2.1. Materials

Three types of poly (lactide-*co*-glycolide), PLGA, with different molecular weights were kindly offered by Mitsui Chemicals Inc. Their weight-averaged molecular weights were about 74 000 (abbreviated to PLGA 75-74 000), 44 000 (PLGA 75-44 000), and 19 000 (PLGA 75-19 000), respectively. The monomer composition of PLGA was 75 mol% lactide and 25 mol% glycolide. 17β-Estradiol was purchased from Tokyo Kasei Kogyo Co., Ltd. Polyvinylalcohol 500 was purchased from Kishida Chemicals.

2.2. Preparation of PLGA microspheres containing estradiol

PLGA microspheres containing 0.3% (w/w) 17β-estradiol were prepared from o/w emulsion by a solvent evaporation process, as reported before [6]. A half gram of PLGA and 0.0015 g of estradiol (0.3% of the polymer weight) were dissolved in 2.0 ml of dichloromethane to make an organic phase. The organic phase was added to 8 ml of 0.3% (w/v) polyvinylalcohol aqueous solu-

tion and mixed by the usage of a vortex mixer for 3 min to prepare o/w emulsion. The o/w emulsion was then poured into 200 ml of 0.3% (w/v) polyvinylalcohol aqueous solution and stirred at 512 rpm for 3 h at room temperature to evaporate dichloromethane. The suspension was centrifuged at 1000 rpm for 5 min and the estradiol-loaded PLGA microspheres precipitated were washed three times with distilled water by centrifugation at 2000 rpm for 5 min. The microspheres were then dried under a reduced pressure and used as a sample in the following experiments. Depending on the type of PLGA used to prepare microspheres, each type of PLGA microspheres is abbreviated to PLGA 75-19 000 MS, PLGA 75-44 000 MS and PLGA 75-74 000 MS, hereafter.

2.3. Degradation properties of PLGA microspheres

Fifty milli grams of PLGA microspheres were redispersed in 5 ml of pH 7.4 phosphate buffer saline and the suspension was kept at 37°C. After a proper time interval, the suspension was centrifuged at 2000 rpm for 5 min and the precipitation was observed under a microscope or dried under a reduced pressure for 1 day for the observation with a scanning electron microscope. The surface properties of PLGA microspheres were observed with a scanning electron microscope (JEOL T-5200).

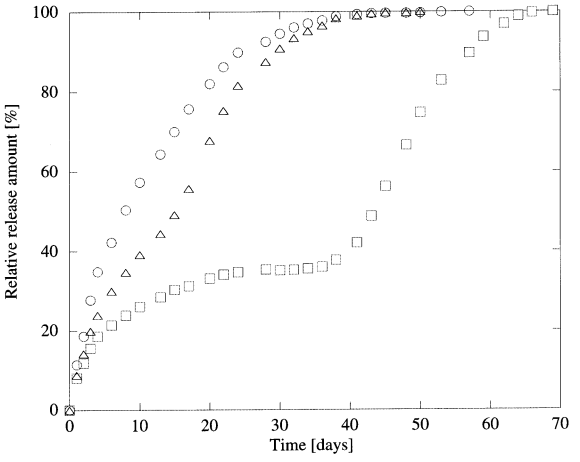

Fig. 1. Release amount of estradiol from PLGA microspheres. 0.3% estradiol was loaded in 75-19 000 MS (○), PLGA 75-44 000 MS (△) and PLGA 75-74 000 MS (□).

2.4. Release of estradiol from PLGA microspheres in vitro

Fifty milligram of PLGA microspheres were redispersed in 5 ml of pH 7.4 phosphate buffer saline and the suspension was kept at 37°C. After a proper time interval, the suspension was centrifuged at 2000 rpm for 5 min. The estradiol concentration in the supernatant was measured with a fluorescence spectrophotometer (Hitachi F-3010) at an excitation wavelength of 268 nm and an emission wavelength of 308 nm.

3. Results and discussion

As reported in a previous paper [6], estradiol release from PLGA microspheres containing 0.3% estradiol was continued almost 3 weeks with an almost constant release rate for 2 weeks after the initial higher rate release, when PLGA of $\overline{M}w$ 23 000 and with the monomer composition of 50% lactide and 50% glycolide was used to prepare the microspheres.

On the other hand, it was newly observed that estradiol was released from PLGA 75-74 000 MS with two stages, as shown in Fig. 1. That is, the release amount of estradiol increases in the initial 30 days up to almost 30%. About 40 days after the release starts, the release amount starts to increase again and the release amount reaches almost 100% 60 days after the degradation starts. Also from PLGA 75-44 000 MS, a similar two-stage release profile to that observed in PLGA75-74 000 MS was observed, while it was less clear compared to that of the latter one. That is, in the initial 15 days, the release amount of estradiol continues to increase up to almost 50%. Twenty days after the degradation starts, the release amount again starts to increase, reaching 100% of release amount 40 days after the release starts. Such a two-stage release of estradiol from PLGA75-19 000 MS was not clearly observed.

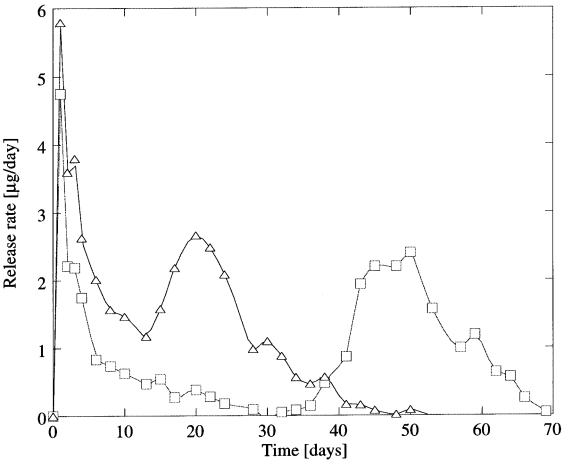

Fig. 2. Release rate of estradiol from PLGA microspheres. 0.3% estradiol was loaded in PLGA 75-44 000 MS (△) and in PLGA 75-74 000 MS (□).

The release rates of estradiol from PLGA 75-44 000 MS and PLGA 75-74 000 MS containing 0.3% estradiol were plotted against time in Fig. 2. It is clearly observed that the release rate of estradiol from PLGA 75-74 000 MS decreases during the first 30 days after the initial burst and then increases again between 30 and 50 days, showing the maximum value 50 days after the degradation starts. On the other hand, the release rate of estradiol from PLGA 75-44 000 MS decreases for about 10 days after the initial burst and then again increases between 12 and 20 days, showing maximum value 20 days after the degradation starts.

Fig. 3 shows microscopic photographs of PLGA 75-74 000 MS under hydrolytic degradation in a pH 7.4 buffer at 37°C. The microspheres begin to swell 35 days after the degradation starts, and their size obviously increases between 42 and 56 days. The surface of the microspheres becomes corrugated 56 days after the immersion and then the microspheres continue to swell even 70 days later. This means that the polymer chains are degraded into those with smaller sizes and then the water content in the matrices increases, which accelerates the hydrolytic degradation rates of PLGA. It should be emphasized here that the release rate of estradiol from PLGA 75-74 000 MS shows a maximum at 50 days after the immersion, and that the shape of the microspheres changes remarkably between 42 and 56 days after the degradation starts. Therefore it is clear that drugs are released from PLGA matrices accompanied with the removal of the degraded segments of the matrices, while they are also released by diffusion of drugs due to their concentration difference between the device interior and the bulk solution.

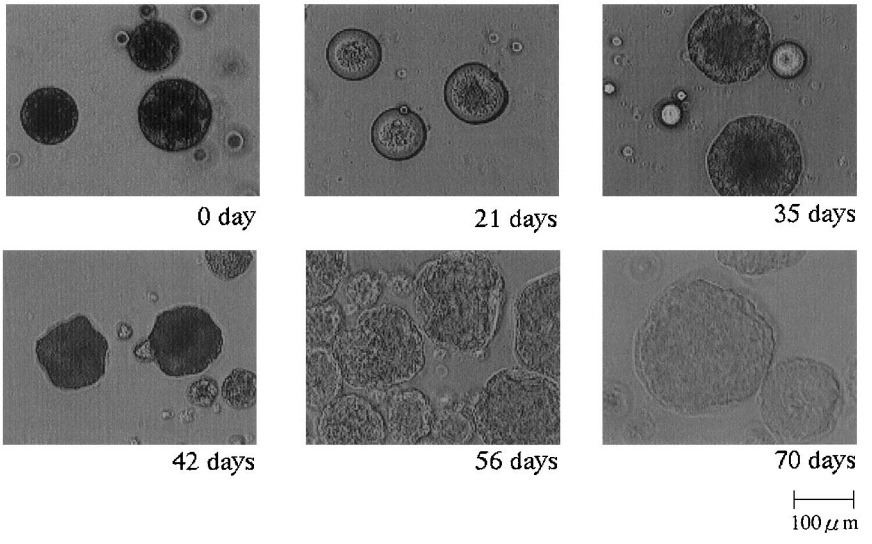

Fig. 3. Microscopic photographs of PLGA 75-74 000 MS at different degradation stages.

Table 1
Mechanisms affecting drug release from PLGA microspheres

1	Dissolution of drugs into polymer matrices and aqueous bulk solution
2	Release of drugs accompanied with the removal of degraded short polymer chains
3	Pore formation in polymer matrices caused by the polymer degradation
4	Changes in water content of PLGA caused by degradation

Therefore, the release rate is considered to be affected by at least four mechanisms shown in Table 1. The relationship between the fourth mechanism and drug release rate is clearly observed by comparison of Figs. 2 and 3. Fig. 4 shows the changes in shape of PLGA 75-44 000 MS caused by the hydrolytic degradation. Also in this type of microspheres, their shape changes most remarkably between 14 and 21 days after the degradation starts, and the microspheres continue to swell between 21 and 42 days. The relationship between the increase in water content of the matrices (the fourth mechanism in Table 1) and the increase in the release rate of estradiol (Fig. 2) is also observed in PLGA 75-44 000 MS. PLGA 75-44 000 MS is completely degraded into monomers within almost 70 days, while it takes more than 70 days for PLGA 75-74 000 MS to be completely degraded.

The third mechanism in Table 1 was confirmed by the observation of microsphere surfaces with SEM. Figs. 5 and 6 show the changes of the surface properties of PLGA 75-74 000 MS and PLGA 75-44 000 MS by the degradation, respectively. A number of fine pores are observed on the surfaces of PLGA 75-74 000 MS 56 days after the degradation starts, while no changes are observed in the initial 28 days, as shown in Fig. 5. On the other hand, PLGA 75-4000 MS have corrugated surfaces with many relatively large pores 28 days after the degradation starts, and then the microspheres change their shapes, becoming sticky 56 days later, as shown in Fig. 6. These observations show the relationship between the second and the third mechanisms in Table 1 and the drug release rate. Therefore, it is concluded that pulsatile estradiol release from PLGA microspheres is mainly controlled by the second and third with the fourth mechanisms.

We have also found that the time interval between the initial burst and the pulsatile release of estradiol can be controlled by mixing PLGA 75-74 000 and PLGA 75-44 000 to prepare the microspheres. Fig. 7 shows the changes of release rate of estradiol from four types of microspheres

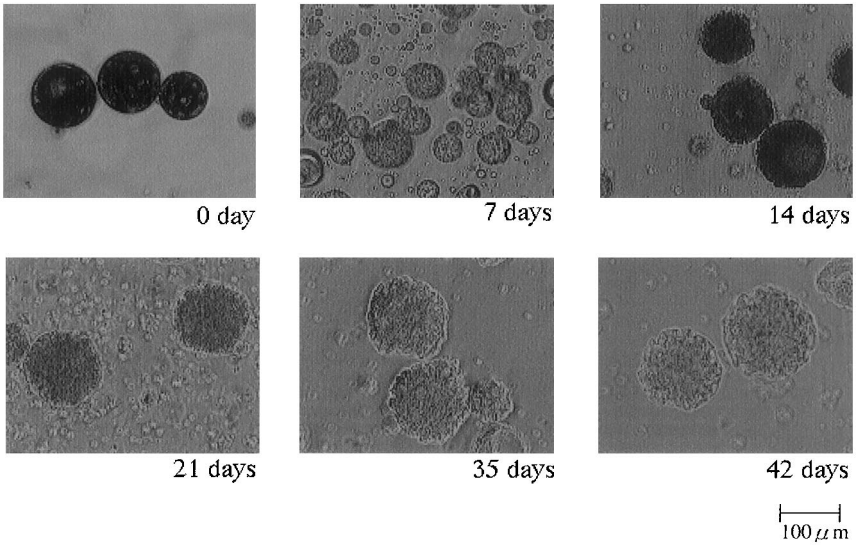

Fig. 4. Microscopic photographs of PLGA 75-44 000 MS at different degradation stages.

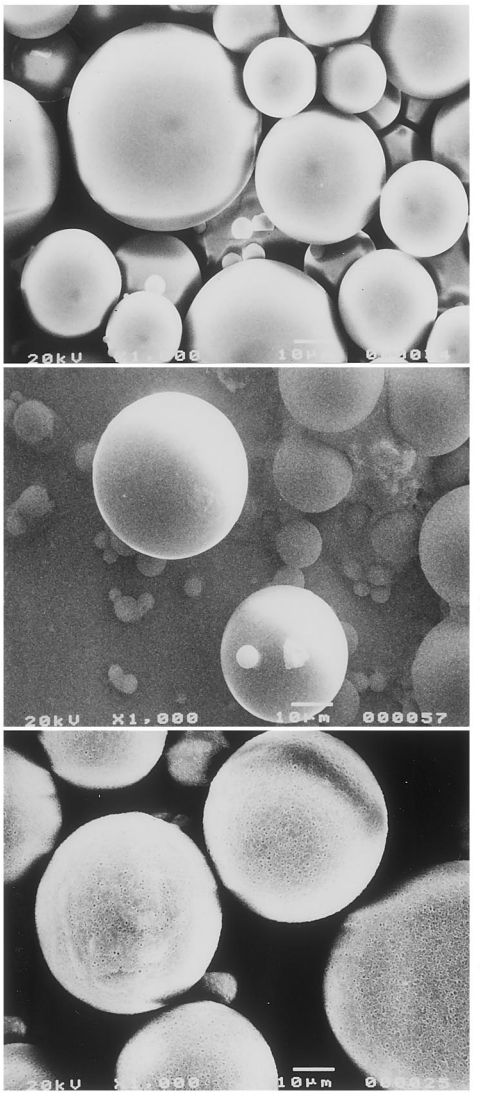

Fig. 5. PLGA 75-74 000 MS surfaces at different degradation stages.

depending on the polymer composition of the microspheres.

Two types of the microspheres shown in Fig. 7 are the same as those shown in Fig. 2 and the other two were obtained by solving two types of PLGA with different ratios in dichloromethane with 0.3% estradiol to prepare oil phase in the preparation process of the microspheres, as mentioned in Section 2. When microspheres are composed of only PLGA 75-44 000 containing 0.3% estradiol, the estradiol release rate shows the maximum value almost 20 days after the degradation starts, as already discussed with Fig. 2. Also when those are composed of only PLGA 75-74 000 containing 0.3% estradiol, the release rate shows the maximum value almost 50 days after the degradation starts, as already discussed with Fig. 2. Interestingly, when the microspheres are composed of 50% (w/w) PLGA 75-44 000 and 50%(w/w) PLGA 75-74 000 and 0.3% estradiol, the

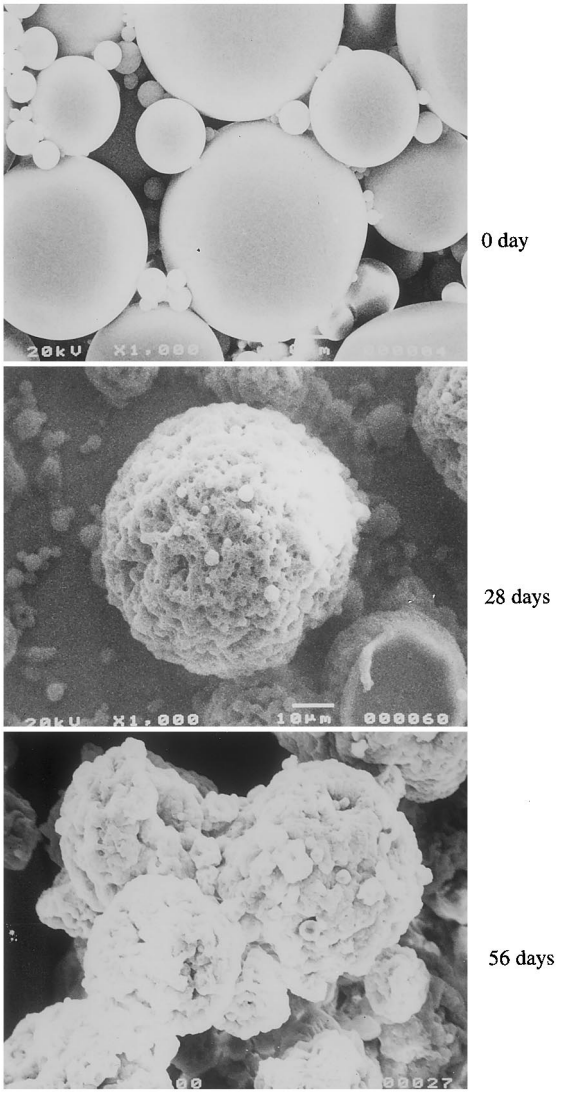

Fig. 6. PLGA 75-44 000 MS surfaces at different degradation stages.

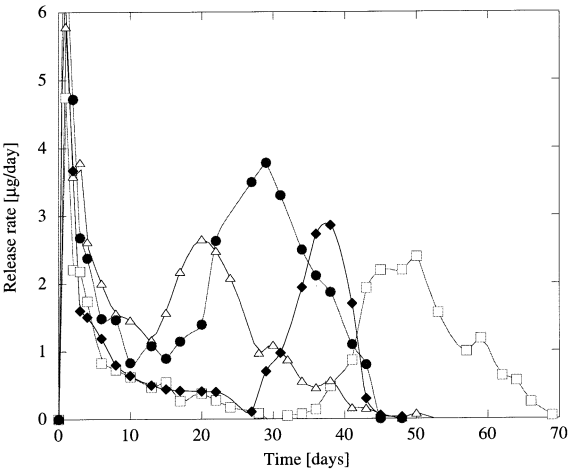

Fig. 7. Release rate of estradiol from PLGA microspheres. 0.3% estradiol was loaded in PLGA 75-44 000 MS (△), in PLGA 75-74 000 MS (□), in the microspheres with the composition of PLGA 75-44 000: PLGA 75-74 000 = 50:50 (●), and in the microspheres with the composition of PLGA 75-44 000: PLGA 75-74 000 = 25:75 (◆).

maximum value of the release rate observed 30 days after the degradation starts, and that when the microspheres are composed of 25% (w/w) PLGA 75-44 000 and 75%(w/w) PLGA 75-74 000 and 0.3% estradiol, the maximum value of the release rate observed 38 days after the degradation starts. Therefore, it is concluded that by the mixture of two types of PLGA with different molecular weights the time interval between the initial burst and the pulsatile release of estradiol can be controlled, since the four mechanisms shown in Table 1 are controlled by the polymer composition of the matrices. Also, we have shown that drug release in a pulsatile manner can be obtained not only from reservoir-type devices like microcapsules [9] but from monolithic-type devices like microspheres composed of biodegradable polymers.

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